Increased vitamin E intake restores fish-oil–induced suppressed blastogenesis of mitogen-stimulated T lymphocytes[1–3]

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ABSTRACT We sought to determine whether fish-oil supplementation would suppress blastogenesis in vitro of concanavalin A (ConA)–stimulated peripheral blood mononuclear cells (PBMCs) and, if so, whether it could be reversed with increased intake of vitamin E. Healthy males ate a controlled basal diet providing a total of 40% of energy from fat when fed in conjunction with 15 g/d of either placebo oil (PO) or fish-oil concentrate (FOC) fortified with 15 mg α-tocopherol/d for three periods. The subjects were supplemented with PO for 10 wk (PO), with FOC for 10 wk (FOC), and with FOC plus an additional 200 mg α-tocopherol/d for 8 wk (FOC+E). During FOC supplementation mitogenic responsiveness of PBMCs to ConA was suppressed, but this effect was reversed by concurrent supplementation with all-*rac*-α-tocopherol (FOC+E). There was a significant positive relationship ($P < 0.001$) between plasma α-tocopherol concentrations and responsiveness of T lymphocytes to ConA. *Am J Clin Nutr* 1991;54:896–902.

KEY WORDS n−3 Polyunsaturated fatty acids, vitamin E, α-tocopherol, fish oil, concanavalin A, T lymphocytes, antioxidant, lymphocyte transformation

Introduction

In recent years there has been increased interest in the proposed health benefits of dietary supplementation with fish oil (1). This interest has resulted in numerous reports showing the effects of n−3 polyunsaturated fatty acids (PUFAs) on health and disease, including those involving the immune system (1–7). Reports describing the effects of n−3 PUFA supplementation on immune functions present mixed results. Studies involving healthy adults eating normal American diets supplemented with n−3 PUFAs demonstrated decreased production of interleukin 1 (IL-1) by peripheral blood mononuclear cells (PBMCs) (2, 3), decreased production of interleukin 2 (IL-2) by T lymphocytes (3), and decreased blastogenesis of mitogen-stimulated PBMCs (3, 4). Bjerve et al (5) showed active mitogenic responsiveness of PBMCs to concanavalin A (ConA) in hospitalized patients with n−3 fatty acid deficiency but, after 4 wk of supplementation with n−3 PUFAs from fish oil, this activity was reduced. In contrast, Payan et al (6) reported increased mitogenic responsiveness of purified T lymphocytes [by formation of rosettes with sheep red blood cells (SRBCs)] to phytohemagglutinin (PHA) from persistent asthmatic patients supplemented with purified eicosapentaenoic acid (EPA) ethyl ester. Kremer et al (7) also reported increased mitogenic responsiveness of PBMCs to ConA from medically treated patients with active rheumatoid arthritis who were supplemented with fish oil.

The increased need for vitamin E during supplementation with n−3 PUFAs (8–10) was taken into consideration in the above studies (3–5). However, these studies (3–5) were not designed to determine the effects of increased intake of vitamin E on mitogen-induced blastogenesis of PBMCs during n−3 PUFA supplementation. Corwin and Shloss (11) demonstrated that mice fed a purified diet containing 8% PUFAs (corn oil) needed a higher intake of vitamin E for maximum blastogenesis of ConA-stimulated spleen lymphoid cells than did mice fed 8% saturated fatty acids (lard or hydrogenated coconut oil). Compared with rats with high stripped-lard intake (10%), rats with high PUFA intake (10% corn oil) showed reduced plasma vitamin E and mitogenic responsiveness of splenic T lymphocytes to ConA, which both were only partially corrected by higher-than-normal intake of vitamin E (200 mg/kg diet) (12).

Given the suppressive effects of n−6 PUFAs on T-lymphocyte responses (13, 14) and the function of vitamin E as an antioxidant (15, 16), the purpose of the present study was to determine whether n−3 PUFA supplementation would suppress mitogenic responsiveness of PBMCs to ConA in vitro and, if so, whether the suppression could be reversed by increased intake of dietary vitamin E.

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[2] Supported in part by funds from a NCI/USDA interagency agreement Y01-CN-40620 and a grant-in-aid from Hoffmann-LaRoche Inc.

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Received October 12, 1990.

Accepted for publication April 10, 1991.

Am J Clin Nutr 1991;54:896–902. Printed in USA.

Subjects and methods

Subjects

Men who were nonsmokers were recruited from the greater Beltsville, MD, area for an initial interview to determine eligibility for entry into the study. The subjects were screened by means of dietary and medical questionnaires designed to exclude those with health problems, such as metabolic disorders; history of any organic disease; regular use of prescription medications or alcohol; and dietary habits that were not representative of the general population.

Those who met the initial selection criteria were examined by a physician from the Georgetown University School of Medicine after having given informed consent according to protocols approved by the Institutional Review Boards of the Georgetown University, the National Cancer Institute (NCI), and the Department of Health and Human Services (DHHS). Part of the medical evaluation before entry consisted of a medical history, a hematologic profile, blood-chemistry measurements including plasma α-tocopherol concentrations, and a urinalysis. Those with body weights $< 90\%$ or $> 120\%$ of the 1983 standards for desirable weights of the Metropolitan Life Insurance Company were excluded from this study (17). Similarly, those with plasma α-tocopheral concentrations < 14 or > 26 μmol/L were also excluded from the study. Aspirin, aspirin-containing drugs, and other anti-inflammatory drugs were not permitted during the study. Acetaminophen was the only analgesic approved for occasional use in cases of acute need. Antibiotics and other medications taken anytime during the study under the direction of a physician were carefully evaluated for their possible effects on study variables. From the initial pool of eligible subjects, 41 were selected for participation in the study; 40 men completed the study. Age, weight, and height were 24–57 y (38.1 ± 1.4, $\bar{x}$ ± SE), 58–118 kg (80.9 ± 2.1), and 169–194 cm (177.6 ± 1.0), respectively.

Diets

A basal diet (**Table 1**) planned from commonly available foodstuffs was designed to contribute a total of 40% of calories from fat when fed in conjunction with 15 g/d of either placebo oil (PO) or fish-oil concentrate (FOC; ROPUFA-50%, containing 50% n−3 fatty acids, Hoffmann-La Roche, Nutley, NJ). The cholesterol intake was ~360 mg/d at 11.7 MJ. The nutrient composition of these diets was calculated by using the US Department of Agriculture (USDA) Lipid Nutrition Laboratory food database, derived mainly from data on food composition from the USDA *Handbook no. 8* and by analysis (18). The content of the long-chain (≥ 20C) and n−3 fatty acids were minimized in the basal diet by excluding fish from the menus whereas vitamin E intake was minimized by excluding highly fortified foods from the diet. A 14-d menu cycle ensured variety and acceptability of the food provided. No smoking, vitamins, minerals, or other supplements, or alcohol were permitted during the study. The meals were prepared in the Human Study Facility of the Beltsville Human Nutrition Research Center (BHNRC) where the subjects came for breakfast and dinner. On weekdays a carry-out lunch was provided whereas weekend meals were packaged and taken home on Fridays. Subjects were initially assigned an appropriate calorie intake based on their estimated need for weight maintenance. Subsequently, body weight was maintained by adjusting the total menu intake by 1.7 MJ (400-kcal) increments. Consumption of coffee, tea, and water was unrestricted.

TABLE 1
Estimated daily nutrient intake on controlled diets*

Nutrient	Placebo	Fish oil
Fat (%)†	40 ± 0.3	40 ± 0.3
Carbohydrate (%)†	46 ± 0.7	46 ± 0.7
Protein (%)†	16 ± 0.3	16 ± 0.3
Cholesterol (mg/d)‡	360 ± 21	360 ± 21
Alpha tocopherol (mg/d, minimum)	22 ± 0.6	22 ± 0.6
Total tocopherol (mg/d, minimum)	41 ± 0.8	41 ± 0.8

* $\bar{x}$ ± SE. Average daily intake for a 14-d menu cycle.
† Percent of energy.
‡ At 11.7 MJ.

All subjects were exclusively fed the controlled basal diet from early January to mid-July for a total of 28 wk divided into three periods. During period 1 (10 wk) all subjects consumed 15 g PO/d (seven capsules at breakfast and eight capsules at dinner) provided as 1-g oil plus 1 mg all-*rac*-α-tocopherol per soft-gelatin capsule (Hoffmann-La Roche). During period 2 (10 wk) all subjects consumed 15 g FOC instead of PO, (seven capsules at breakfast and eight capsules at dinner) each capsule containing 1 mg all-*rac*-α-tocopherol. During period 3 (8 wk) all subjects continued to consume FOC capsules but at breakfast also received a capsule containing 200 mg all-*rac*-α-tocopherol. Total α-tocopherol intake from the diet plus the capsules (PO or FOC) was a minimum of 22 mg/d.

The PO was a blend of 48% stripped lard, 40% beef tallow (Canada Packers, Toronto; hormone-free), and 12% corn oil, 1 g per capsule, stabilized with 1 mg all-*rac*-α-tocopherol. The n−3 fatty acid supplement was a 50% concentrate of refined anchovy oil (ROPUFA-50%). This supplement, as 1 g per soft-gelatin capsules (containing 1 mg all-*rac*-α-tocopherol) was identical in appearance to the PO capsule. Subjects were informed of the sequence of administration of the capsules.

Estimated total daily nutrient intake and fatty acid intake are presented in **Table 1** and **Table 2.** Composition of the PO and FOC supplements, determined by capillary gas chromatography as previously described (19), are shown in **Table 3.**

Collection of blood and mononuclear blood cells

At the end of each dietary period, 20 mL fasting blood was collected from the subjects by venous puncture. The blood was collected in 10-mL sterile evacuated tubes to which 500 U heparin (Sigma Chemical Co, St. Louis) had been added under sterile conditions. The blood was collected between 0630 and 0830 and held at room temperature until being processed at 0900. The tubes of blood from each subject were emptied into a 50-mL conical tube (Falcon, Becton Dickinson, Lincoln Park, NJ) and centrifuged at 1000 × *g* for 10 min at 20 °C. The plasma was removed with a sterile transfer pipette (Sarstedt Inc, Newton, NC) and placed in a 15-mL conical tube. The plasma was recentrifuged at 1000 × *g* for 10 min at 4 °C, removed from pelleted residual blood cells, and held at 4 °C for ~4 h before being used as autologous plasma (AP) in the culturing of PBMCs.

TABLE 2
Estimated daily fatty acid intake on controlled diets*

Fatty acids	Placebo	Fish oil
	g/d†	
Total saturates	33 ± 1.1	29 ± 1.1
Palmitic (16:0)	20 ± 0.6	18 ± 0.6
Stearic (18:0)	9 ± 0.3	7 ± 0.3
Total monounsaturates	46 ± 0.6	42 ± 0.6
Oleic (18:1n−9)	44 ± 0.6	39 ± 0.6
Total polyunsaturates	27 ± 0.6	33 ± 0.6
n−3 Polyunsaturates	2 ± 0.1	10 ± 0.1
Linolenic acid (18:3n−3)	2 ± 0.1	2 ± 0.1
Eicosapentaenoic acid (20:5n−3)	0	5 ± 0.0
Docosahexaenoic acid (22:6n−3)	0	2 ± 0.0
n−6 Polyunsaturates	25 ± 0.6	23 ± 0.6
Linoleic acid (18:2n−6)	25 ± 0.6	23 ± 0.6

* $\bar{x}$ ± SE.
† At 11.7 MJ

The packed blood cells were resuspended to original blood volume with 1× Hanks' Balanced Salt Solution (HBSS, GIBCO Laboratories, Grand Island, NY) containing penicillin and streptomycin (GIBCO) at 100 kU/L and 100 mg/L, respectively.

PBMCs were prepared by gradient centrifugation. Ten milliliters of the resuspended blood cells were placed on 4 mL Histopaque-1077 (Sigma Chemical) in a 15-mL conical tube. The tubes were centrifuged at 400 × *g* for 30 min at 20 °C. Recovered PBMCs were washed twice with HBSS by centrifugation at 1000 × *g* for 10 min at 10 °C. After the second centrifugation the pelleted PBMCs were resuspended in RPMI-1640 tissue-culture medium (GIBCO) containing (per L) 2.0 mmol L-glutamine (GIBCO), 100 kU penicillin, and 100 mg streptomycin, referred to herein as RPMI-1640. Viable PBMCs were determined by trypan blue exclusion and adjusted to 4×10^9 cells/L in RPMI-1640.

Lymphocyte mitogenic assay

In vitro cell cultures received, in order, 50 μL of adjusted PBMCs (2×10^5 cells/well), 50 μL RPMI-1640 alone (background) or RPMI-1640 containing ConA (type IV, Sigma Chemical Co), and 100 μL of RPMI-1640 containing 20% (10% final concentration) heat-inactivated (56 °C for 30 min) fetal bovine serum (FBS, GIBCO) or noninactivated AP per well of 96-well microtiter plates. Each cell culture contained a total volume of 200 mL. Stock solutions of FBS and ConA were portioned and stored at −20 °C before study was begun. Single lots and uniform storage of all tissue-culture reagents were used throughout the study.

Triplicate background (without mitogen) cultures were evaluated for each cell-culture sample; triplicate test (with ConA) cultures were likewise evaluated for each dose of ConA tested. To determine the mitogen dose-response curve, ConA was added at concentrations of 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, and 25.6 μg per culture. The cell cultures were incubated for 72 h at 37 °C in an incubator with 5% CO_2 and 95% humidified air. Twenty-four hours before termination of incubation, 1.0 kBq [methyl-^{3}H]thymidine (specific activity 248 GBq/mmol; Dupont NEN Products, Boston) was added to each culture. On completion of incubation the cells were harvested with a Titertek Cell Harvester (Skatron Inc, Sterling, VA). The thymidine-labeled DNA was collected on 12-well filtermats (Skatron). Individual filter discs were placed in plastic Mini-Poly Vials (Beckman, Fullerton, CA), 5 mL Ready Solve HP/b (Beckman) was added to each vial, and the samples were counted in a Beckman LS 3801 scintillation counter by using a single-label dpm program (1 Bq = 1.0 dps).

Fatty acids and α-tocopherol analyses

The fatty acid composition of platelets and the α-tocopherol composition of plasma, platelets, and erythrocytes were determined as previously described by other investigators from this laboratory (20).

Statistics

Individual scintillation counts for each triplicate set of background (without ConA) and test (with ConA) cultures were analyzed as follows to determine whether any counts should be rejected as outliers. For each culture type (AP, FBS), supplementation (PO, FOC, FOC+E), and ConA concentration (0.0, 0.8, 1.6, 3.2, 6.4, 12.8) an analysis-of-variance model was fit for subject effects. Residuals (deviations of observed counts from the subjects mean count) for this model were compared with the SD and the interquartile range. If an absolute value of the residual was greater than three times the SD or the interquartile range (center 50% of the data) of the residuals, the corresponding

TABLE 3
Fatty acid composition of placebo and fish-oil supplements

Fatty acid	Placebo	Fish oil
	g/100 g	
12:0	0.2	0.1
14:0	2.1	4.9
16:0	21.8	9.3
16:1n−7	2.4	6.5
18:0	13.3	1.4
18:1n−9	36.4	5.4
18:2n−6	13.7	1.9
18:3n−3	0.4	1.0
18:4n−3	ND*	4.6
20:1n−9	0.4	0.4
20:4n−6	0.2	1.4
20:4n−3	ND	1.1
20:5n−3	ND	30.2
22:5n−3	ND	2.5
22:6n−3	ND	13.1
Saturates	38.4	16.5
Monounsaturates	42.8	15.7
Polyunsaturates	14.4	63.6
n−6	13.9	3.7
n−3	0.4	52.5
n−3:n−6†	0.03	14.16

* Not detected.
† Ratio of n−3 to n−6 fatty acids.

observed count was deleted. Of the 3819 observed counts, 99 were identified as outliers and removed from the triplicate data set. The mean of the remaining counts for each subject, culture type, supplementation, and ConA concentration was used in subsequent analyses.

Analyses were carried out on mean values computed for each combination of subject, culture type, supplementation, and ConA concentration. Counts difference (CD) was computed as the difference between the observed mean count at each ConA concentration and the mean count at the zero ConA concentration.

Univariate analyses of variance were used to test the effects of supplementation, ConA, and the interaction of supplementation by ConA for each culture type. The model used also included the effects of subject and a subject-by-supplementation interaction to be used as the error variance for tests of hypotheses about supplementation, whereas the residual error variance was used for tests of ConA and the supplementation by ConA interaction. The inclusion of two error variances in the model accounted for the greater error variance expected among periods than would be expected to occur within periods. These two error variances were used to compute appropriate SEs as required in split-plot designs to conduct mean comparisons by using the least-significant-difference test (21).

Residuals from this model were tested for outliers, as above, and examined to determine if the assumptions of the normality and homogeneity of variances were reasonable for the parametric analysis of variance. Residual plots for examining normality and Levene's test of homogeneity of variances were used (22). Neither assumption was reasonable for the variable CD. Examination of the residual plot suggested that a log transformation might prove satisfactory. After logarithmic transformation of CD data, a second examination of the model residuals indicated that the transformation had successfully improved the normality assumption. Of the 1060 subjects, culture type, supplementation, and ConA combination means, nine means (0.85% of total) were removed as outliers at this step. However the assumption of homogeneity of variances was still untenable. To account for the variance heterogeneity, the final analysis of variance was weighted for the reciprocal of the residual variances for each culture type, supplementation, and ConA concentration combination. That is, those treatment combinations with the greatest variance received the smallest weights whereas those with the smallest variance were assigned the largest weights in the analysis of variance (23).

Results

EPA and α-tocopherol profiles of subjects were determined after each period of supplementation. Mean weight percent of EPA (20:5n−3) in platelet membranes increased from 0.2% after supplementation with PO to 4.1% and 4.2% after supplementation with FOC and FOC+E, respectively. The ratio of n−3 to n−6 PUFAs in platelets increased from 0.08 after supplementation with PO to 0.35 after FOC and FOC+E. Concentration ($\bar{x} \pm$ SE) of α-tocopherol in plasma significantly decreased from 24 ± 0.71 to 20 ± 0.71 μmol/L after supplementation with FOC and significantly increased to 26.8 ± 0.94 μmol/L after supplementation with FOC+E. Similar significant changes of α-tocopherol were found in platelets and erythrocytes (data not shown).

Mitogenic responsiveness to ConA of PBMCs cultured in medium containing either FBS or AP is shown in **Figures 1 and 2,** respectively. Activities of cells stimulated with extremely suboptimal (0.2 and 0.4 μg) and excess (25.6 μg) concentrations of ConA are not presented. PBMCs from the subjects after supplementation with FOC showed suppressed blastogenesis when cultured in medium containing FBS and stimulated with a wide range of ConA concentrations (Fig 1). The suppressed blastogenesis was restored after supplementation of the subjects with an additional 200 mg all-*rac*-α-tocopherol/d during the third period (FOC+E).

PBMCs from FOC-compared with PO-supplemented subjects showed reduced blastogenesis when cultured in medium containing AP and stimulated with concentrations of ConA in excess (6.4 and 12.8 μg) of that needed to induce maximum mitogenic responsiveness (Fig 2). The suppressed mitogenic response to ConA was corrected after supplementation of the subjects with FOC+E. PBMCs from the subjects after each supplementation showed equivalent mitogenic responsiveness when cultured in medium containing AP and stimulated with concentrations of ConA (0.8, 1.6, and 3.2 μg) that induced optimal blastogenesis. Mitogenic responsiveness of PBMCs to the complete range of ConA concentrations was equivalent for cells from subjects after supplementation with PO or FOC+E.

Regression analysis was used to assess the relation between log CD (AP and FBS) and plasma α-tocopherol within subjects because subjects were sampled in both periods. There was a significant positive relationship ($P < 0.001$) for both AP and FBS with plasma α-tocopherol. The regression coefficients were $+3.06 \pm 0.79$ and $+3.16 \pm 0.71$ for log CD regressed on plasma α-tocopherol for AP and FBS, respectively.

Discussion

Subjects in the present study supplemented with FOC for 10 wk showed significantly increased EPA in platelet membranes but significantly reduced plasma platelet, and erythrocyte α-tocopherol (data not shown). After continued supplementation for 8 wk with FOC+E, at 22 times the RDA, then n−3 PUFA concentration of EPA in platelet membranes remained the same and the amount of α-tocopherol in plasma, platelets and erythrocytes significantly increased. On the basis of significant concentration changes of α-tocopherol in plasma, platelet, and erythrocyte specimens, we assume that similar changes occurred in PBMCs. Hatam and Kayden (24) and Meydani et al (25) observed a two-to-threefold increase of α-tocopherol in plasma and PBMCs from subjects supplemented with amount not reported and 800 mg vitamin E/d, respectively.

Our finding of suppressed mitogenic responsiveness of PBMCs stimulated with ConA from FOC-supplemented subjects agrees with reports describing suppressed blastogenesis of mitogen-stimulated PBMCs from subjects supplemented with n−3 PUFA (3–5). Results of our study, however, indicate that the suppression was associated with a reduced α-tocopherol status. PBMCs from FOC-supplemented subjects showed suppressed mitogenic responsiveness to ConA at a wide range of concentrations when cultured in medium containing FBS but only to ConA in excess

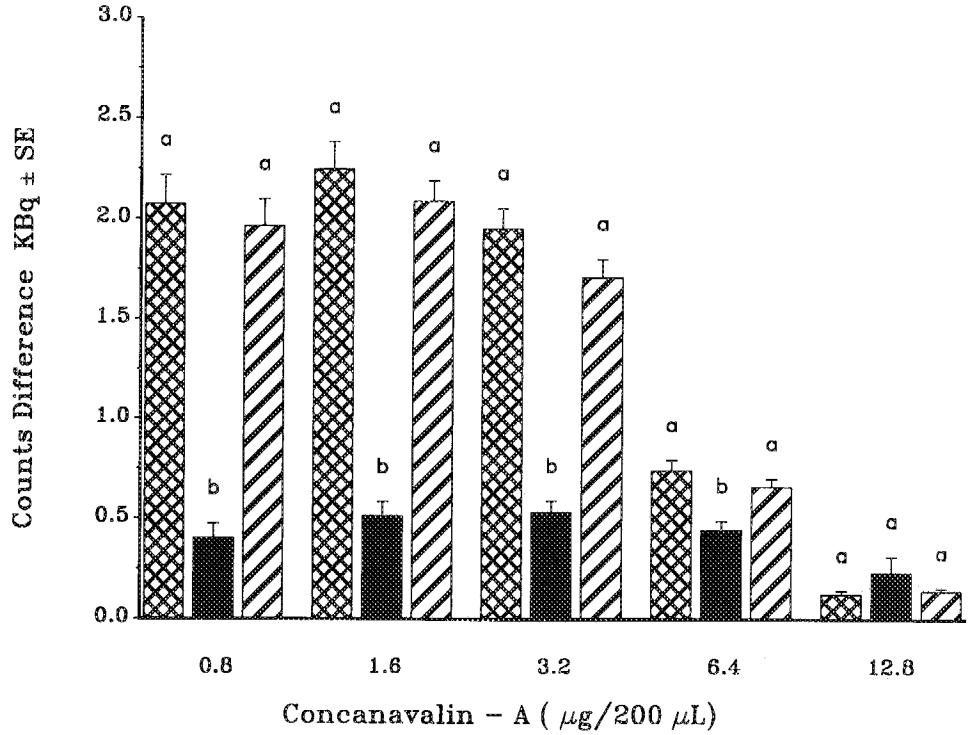

FIG 1. Blastogenesis ([^{3}H]thymidine incorporation) of ConA-stimulated PBMCs from subjects after supplementation with PO (▨), FOC (▩), or FOC+E (▨), cultured in medium containing FBS. Mitogenic responsiveness expressed as counts difference (stimulated minus unstimulated counts) kBq. $\bar{x}$ + SE, n = 34–39. Dissimilar letters denote significant differences ($P < 0.05$) between values within a ConA concentration by least-significant difference.

of that needed for maximum blastogenesis when cultured in medium containing AP (Figs 1 and 2). The mean concentration of α-tocopherol in standard commercially available FBS is < 1.0 μmol/L (information from Hyclone Laboratories, Salt Lake City) whereas that in plasma from FOC subjects was 20 ± 0.7 μmol/L ($\bar{x}$ ± SE). The suppressed blastogenesis was reversed in PBMCs from subjects supplemented with additional vitamin E (FOC+E). Because significantly higher concentrations of α-tocopherol were achieved in plasma, platelets, and erythrocytes of FOC+E than of FOC subjects, it is concluded that supplementation with additional vitamin E reversed the FOC-induced decline in plasma vitamin E status. Because the concentrations of fatty acids and α-tocopherol in FBS remained constant, it is assumed that enhanced blastogenesis of PBMCs stimulated with ConA from FOC+E subjects was related to an increased cellular concentration of α-tocopherol. This assumption is supported by mitogenic responsiveness of T lymphocytes to ConA showing a statistically significant correlation by regression analysis with the rise in plasma α-tocopherol concentrations.

The dietary amount of vitamin E needed for maximum blastogenesis of mitogen-stimulated PBMCs from individuals consuming increased amounts of n−3 PUFAs is not established. The present study and that of Kelly et al (4) may indicate that it is higher than the current RDA of 10 tocopherol equivalents/d for adult males and females (10). Also using in vitro PBMC cultures supplemented with FBS, Kelly et al (4) demonstrated only marginally significant reduced blastogenesis of PBMCs stimulated with ConA from healthy men supplemented with flaxseed oil plus 200 mg all-*rac*-α-tocopherol/wk for 8 wk in addition to that acquired from the basal diet. PBMCs from FOC subjects of this study, supplemented with approximately half that amount of all-*rac*-α-tocopherol per week (105 mg), showed significantly suppressed blastogenesis of PBMCs stimulated with ConA. After supplementation with 1505 mg all-*rac*-α-tocopherol/wk for 8 wk, the subjects (FOC+E) showed restored blastogenesis of PBMCs stimulated with ConA (Fig 1). Information is not available describing the minimum amount of extra α-tocopherol needed to reverse the suppressive effects of n−3 PUFAs on blastogenesis of human PBMCs stimulated with ConA. Bendich et al (26) demonstrated, in rats fed a basal diet containing 10% stripped lard (vitamin E free) plus increasing amounts of vitamin E, that the vitamin E required for maximum blastogenesis of spleen lymphoid cells stimulated with ConA was more than three times higher (15 vs 50 mg/kg) than that needed for other measurements of vitamin E adequacy.

In contrast to our findings and those of others (3, 4), Payan et al (6) and Kremer et al (7) observed increased blastogenesis of mitogen-stimulated lymphocytes from n−3 PUFA–supplemented, medically treated patients with asthma and rheumatoid arthritis respectively. The difference in responsiveness observed by Payan et al (6) may be partially explained by either the drug usage of medically treated asthmatic patients or the method of T-lymphocyte isolation before stimulation in vitro with PHA. They isolated T lymphocytes from PBMCs by the formation of rosettes with SRBCs. Breitmeyer and Faustman (27) demonstrated enhanced blastogenesis of purified human T lymphocytes stimulated with PHA after isolation by formation of rosettes with SRBCs.

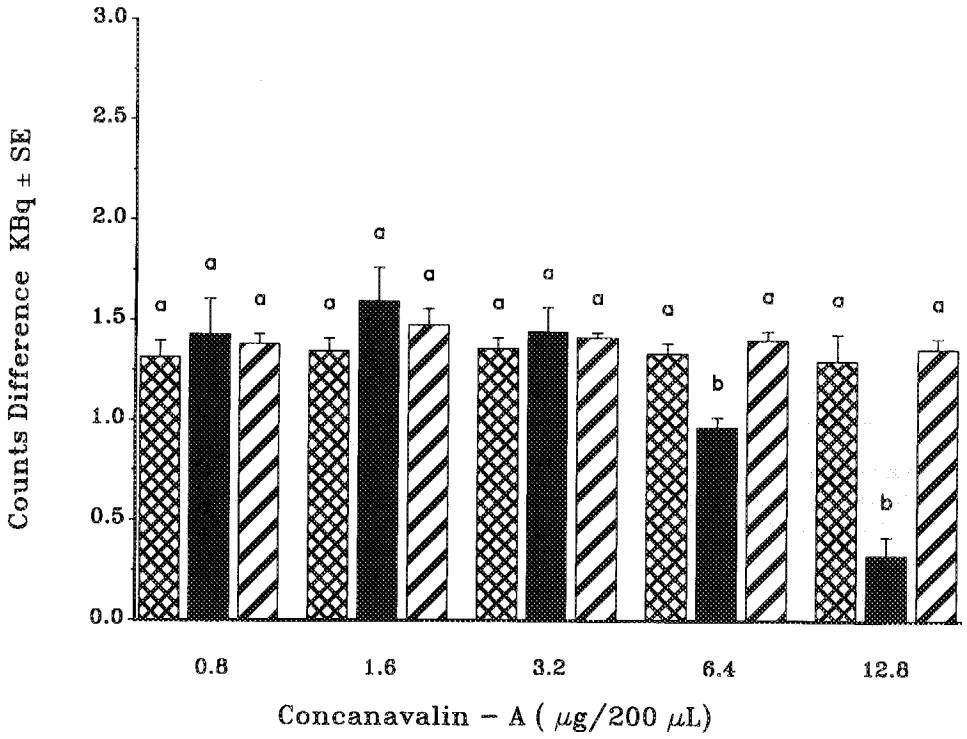

FIG 2. Blastogenesis ([^{3}H]thymidine incorporation) of ConA-stimulated PBMCs from subjects after supplementation with PO (▨), FOC (■), FOC+E (▨), cultured in medium containing AP. Mitogenic responsiveness expressed as counts difference (stimulated minus unstimulated counts) kBq. $\bar{x}$ + SE, n = 34–39. Dissimilar letters denote significant differences ($P < 0.05$) between values within a ConA concentration by least-significant difference.

Our results indicate that the amount of dietary vitamin E needed for maximum blastogenesis in vitro of PBMCs stimulated with ConA is elevated with increased dietary intake of n−3 PUFAs. Additional studies are needed to determine how much of an increase in dietary vitamin E is required for optimal immune protection by the T-lymphocyte division of the immune system in individuals consuming increased amounts of n−3 PUFAs. Individuals with already impaired T-lymphocyte functions should be aware of a possible need for increased intake of vitamin E during prolonged dietary supplementation with fish oil. It may be important to examine whether vitamin E will be beneficial in other conditions with suppressed T-lymphocyte function. ■

We thank Priscilla Steele and the entire staff of the Human Study Facility of the Beltsville Human Nutrition Research Center for efforts in meal preparation for the controlled diets; W Campbell, US Public Health Service, and M Sunkin for keeping medical records, for recruiting, instructing, and supervising the subjects, and for blood drawing; Maria Horsey for technical and computer assistance; and Mark Rue for assistance in installing computer collection of scintillation counts.

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